

What is Claimed Is:

1. A method for the production of an antibody by an avian cell comprising culturing an avian cell transfected with at least one expression vector
5 comprising a transcription unit having a nucleotide sequence encoding an immunoglobulin polypeptide operably linked to a transcription promoter and a transcriptional terminator, and wherein the cultured avian cell produces an immunoglobulin polypeptide capable of forming an antibody.
- 10 2. The method of Claim 1, wherein the immunoglobulin polypeptide is an immunoglobulin heavy chain variable region, an immunoglobulin heavy chain variable region and a constant region, an immunoglobulin light chain variable region, an immunoglobulin light chain variable region and a constant region and a single-chain antibody comprising two linked immunoglobulin variable
15 regions.
3. The method of Claim 1, wherein the at least one expression vector further encodes a second immunoglobulin polypeptide and an internal ribosome entry site (IRES).
- 20 4. The method of Claim 1, wherein the immunoglobulin polypeptide has a peptide region suitable for the isolation of the immunoglobulin polypeptide.

5. The method of Claim 1, wherein the avian cell is derived from a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.

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6. The method of Claim 1, wherein the avian cell is selected from a fibroblast, an oviduct cell, an ovum, a testicular cell, and an embryonic cell.

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7. The method of Claim 6, wherein the avian cell is an oviduct cell or an embryonic cell.

8. The method of Claim 1, wherein the avian cell is cultured *in vivo* in an avian species selected from a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.

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9. The method of Claim 1, wherein the at least one expression vector is selected from a viral vector, a plasmid vector, a linear nucleic acid vector, or a combination thereof.

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10. The method of Claim 9, wherein the at least one expression vector is a viral vector selected from the group comprising avian leukosis virus, adenoviral vectors, transferrin-polylysine enhanced adenoviral vectors, human

immunodeficiency virus vectors, lentiviral vectors, Moloney murine leukemia virus-derived vectors or variants thereof.

11. The method of Claim 9, wherein the at least one expression vector is a plasmid
5 vector.
12. The method of Claim 1, wherein the transcriptional promoter of the at least one expression vector is a constitutively active promoter.
- 10 13. The method of Claim 12, wherein the transcriptional promoter of the at least one expression vector is a cytomegaloviral promoter.
14. The method of Claim 1, wherein the transcriptional promoter of the at least one expression vector is a tissue-specific promoter.
- 15 15. The method of Claim 14, wherein the tissue-specific promoter is operable in oviduct cells of an avian species.
16. The method of Claim 15, wherein the tissue-specific promoter is selected from
20 the promoters of the genes encoding ovalbumin, lysozyme, ovomucoid, ovotransferrin (conalbumin), and ovomucin.

17. The method of Claim 1, wherein the transcriptional promoter of the at least one expression vector is a regulatable promoter.
18. The method of Claim 1, wherein the transcriptional terminator of the at least one expression vector comprises a region encoding a bovine growth hormone transcriptional terminator.
19. The method of Claim 1, wherein the immunoglobulin polypeptide encoded by the transcriptional unit of the at least one expression vector is an immunoglobulin heavy chain variable region or a variant thereof.
20. The method of Claim 19, wherein the immunoglobulin heavy chain further comprises a D region, a J region and a C region.
21. The method of Claim 1, wherein at least one immunoglobulin polypeptide encoded by the transcriptional unit of at least one expression vector is an immunoglobulin light chain variable region or a variant thereof.
22. The method of Claim 21, wherein the immunoglobulin light chain further comprises a J region and a C region.

23. The method of Claim 19, wherein the immunoglobulin polypeptide is a mammalian or an avian immunoglobulin heavy chain polypeptide.
24. The method of Claim 23, wherein the immunoglobulin heavy chain polypeptide comprises at least two domains derived from at least two animal species.
25. The method of Claim 23, wherein the mammal is a human, a mouse, a rat, a rabbit, a goat, a sheep, a cow or a horse, and wherein the avian is a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.
26. The method of Claim 1, wherein the immunoglobulin polypeptide is a mammalian or an avian immunoglobulin light chain polypeptide.
27. The method of Claim 26, wherein the immunoglobulin polypeptide comprises at least two domains derived from at least two animal species.
28. The method of Claim 26, wherein the mammal is a human, a mouse, a rat, a rabbit, a goat, a sheep, a cow or a horse, and wherein the avian is a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.

29. The method of Claim 1, wherein the immunoglobulin polypeptide encoded by the transcriptional unit of at least one expression vector comprises an immunoglobulin heavy chain variable region, an immunoglobulin light chain variable region, and a linker peptide, and thereby forming a single-chain antibody.
30. A method for the production in an avian of an heterologous immunoglobulin polypeptide, comprising the steps of:
- (a) producing a transgenic avian comprising at least one transgene encoding at least one heterologous immunoglobulin polypeptide;
 - (b) expressing the at least one heterologous immunoglobulin polypeptide in a tissue of the transgenic avian; and
 - (c) isolating the at least one heterologous immunoglobulin polypeptide from the avian tissue.
31. The method of Claim 30, wherein the avian tissue is serum or the white of a developing avian egg.
32. The method of Claim 30, wherein the transgene comprises a transcription unit encoding a first immunoglobulin polypeptide and a second immunoglobulin

polypeptide operably linked to a transcription promoter, a transcription terminator, and optionally an internal ribosome entry site (IRES).

33. The method of Claim 30, wherein the transgenic avian expresses a first and a
5 second transgene encoding a first and a second heterologous immunoglobulin polypeptides, and wherein the method further comprises the step of combining the first and second heterologous immunoglobulin polypeptides, thereby forming an antibody.
- 10 34. The method of Claim 33, wherein the antibody comprises at least one immunoglobulin heavy chain variable region and at least one immunoglobulin light chain variable region.
- 15 35. The method of Claim 34, wherein the immunoglobulin heavy chain polypeptide is a mammalian or an avian immunoglobulin heavy chain polypeptide.
- 20 36. The method of Claim 35, wherein the mammal is a human, a mouse, a rat, a rabbit, a goat, a sheep, a cow or a horse, and wherein the avian is a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.

37. The method of Claim 35, wherein the immunoglobulin light chain polypeptide is a mammalian or an avian immunoglobulin light chain polypeptide.
38. The method of Claim 37, wherein the mammal is a human, a mouse, a rat, a rabbit, a goat, a sheep, a cow or a horse, and wherein the avian is a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.
39. The method of Claim 30, wherein the transgenic avian is produced by sperm-mediated transfer of a transgene or by introducing a transgenic avian donor nucleus into a recipient cell to produce a reconstructed avian zygote, activating the reconstructed zygote and allowing the reconstructed zygote to develop to term.
40. The method of Claim 30, wherein the transgenic avian is produced by sperm-mediated transfer of at least one transgene, wherein the at least one transgene is incorporated into the spermatozoan cell or a precursor thereof, so that a genetically modified male gamete is produced by the male avian; and breeding the male avian with a female of its species such that a transgenic progeny is produced that carries the at least one transgene in its genome.

41. The method of Claim 30, further comprising the steps of enucleating a recipient cell, introducing a transgenic avian donor nucleus into the enucleated recipient cell to produce a reconstructed avian zygote, activating the reconstructed zygote and allowing the reconstructed zygote to develop to term.

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42. The method of Claim 41, wherein the recipient cell is enucleated using two photon laser scanning microscopy.

43. The method of Claim 41, wherein the transgenic avian donor nucleus is obtained from a transgenic avian cell, wherein the transgenic avian cell comprises a transgene encoding at least one heterologous immunoglobulin polypeptide.

44. The method of Claim 41, further comprising integrating the transgene into the genomic DNA of an avian sperm by an *in vivo* method, comprising the steps of:

(a) administering to a avian testis a gene delivery mixture comprising a viral vector having at least one heterologous polynucleotide encoding at least one heterologous immunoglobulin polypeptide, the heterologous polynucleotide being operatively linked to a transcriptional promoter, under conditions effective to reach a spermatozoan cell or a precursor

cell within the testis, the precursor cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, and spermatids;

- (b) incorporating the heterologous polynucleotide encoding the at least one heterologous polypeptide into the genome of the spermatozoan cell or the precursor cell, so that a genetically modified male gamete is produced by the male avian; and
- (c) breeding the male avian with a female of the same species such that a transgenic progeny is thereby produced that carries the heterologous polynucleotide in its genome.

45. The method of Claim 44, wherein the vector further comprises a second transgenic polynucleotide sequence encoding a immunoglobulin polypeptide and an internal ribosome entry sequence (IRES) operably linked thereto.

46. The method of Claim 41, wherein the transgene is integrated into the genomic DNA of an avian sperm by an *in vitro* method, comprising the steps of:

- (a) obtaining from a donor male avian a spermatozoan cell or a precursor cell, the precursor cell being selected from the group

consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, and spermatids;

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(b) genetically modifying the spermatozoan cell or precursor cell *in vitro* with at least one heterologous polynucleotide encoding at least one heterologous polypeptide, the heterologous polynucleotide being operatively linked to a promoter sequence such that a transcriptional unit is formed, and a gene encoding a genetic selection marker, in the presence of a gene delivery mixture comprising a viral vector, and for an effective period of time such that the transcription unit is integrated into the genome of the cell;

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(c) isolating or selecting the genetically modified cell with the aid of the genetic selection marker expressed in the genetically modified cell;

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(d) transferring the thus isolated or selected genetically modified cell of step (c) to a testis of a recipient male avian such that the cell lodges in a seminiferous tubule of the testis and a genetically modified male gamete is produced therein: and

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(e) breeding the recipient male avian with a female avian of its species such that a transgenic progeny is thereby produced that

carries the heterologous polynucleotide in its genome.

47. The method of Claim 46, wherein step (b) further includes a transgenic second polynucleotide sequence encoding an immunoglobulin polypeptide and an internal ribosome entry sequence (IRES) for genetically modifying the spermatozoan cell or precursor cell.

48. The method of Claim 44, wherein the spermatozoan cell or precursor cell is genetically modified by incorporating a transgene into an avian sperm or an avian nucleus by restriction enzyme mediated integration (REMI) comprising the steps of

(a) administering to a avian sperm cell or a precursor sperm cell a gene delivery mixture comprising at least one heterologous polynucleotide encoding at least one heterologous immunoglobulin polypeptide, the heterologous polynucleotide being operably linked to a promoter sequence such that a transcriptional unit is formed;

(b) forming cohesive ends on the heterologous polynucleotide such that the cohesive ends are identical to the cohesive ends characteristic of a DNA cleaved by a given type II restriction endonuclease;

transferring the heterologous polynucleotide having cohesive ends, and

the type II restriction endonuclease to a spermatozoan cell or a precursor cell, the precursor cell being selected from the group consisting of spermatogonial stem cells, type B spermatogonia, primary spermatocytes, preleptotene spermatocytes, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes, secondary spermatocytes, and whole viable avian sperm, thereby genetically modifying male gametes as produced by the male avian.

10 49. The method of Claim 46, wherein the avian is selected from a chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental bird or a feral bird.

50. The method of Claim 46, wherein the avian is a chicken.

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51. A transgenic avian comprising at least one heterologous polynucleotide sequence encoding at least one heterologous immunoglobulin polypeptide.

52. The transgenic avian of Claim 51, wherein the immunoglobulin polypeptide is delivered to the white of an avian egg by a female of the transgenic avian.

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53. The transgenic avian of Claim 51, wherein the immunoglobulin polypeptide is delivered to the serum of the transgenic avian.
54. The transgenic avian of Claim 51, wherein the at least one heterologous polynucleotide sequence further comprises a nucleotide sequence, a transcription promoter and a transcriptional terminator operably linked to the nucleotide sequence encoding the at least one immunoglobulin polypeptide.
55. The transgenic avian of Claim 54, further comprising an internal ribosome entry site (IRES) operatively linked to a nucleotide sequence encoding at least one immunoglobulin polypeptide.
56. The transgenic avian of Claim 54, wherein the transgenic avian is produced according to the method of Claim 40.
57. The transgenic avian of Claim 54, wherein the transgenic avian is produced by nuclear transfer integration of the at least one heterologous nucleic acid sequence according to the method of Claim 41.
58. The transgenic avian of Claim 54, wherein the transgenic avian is produced by sperm-mediated integration of the at least one heterologous nucleic acid

sequence according to the restriction enzyme mediated integration method of Claim 41.

59. The transgenic avian of Claim 54, wherein the transgenic avian is produced by
5 sperm-mediated integration of the at least one heterologous nucleic acid
sequence according to the restriction enzyme mediated integration method of
Claim 46.

60. The transgenic avian of Claim 51, wherein the avian is selected from a
10 chicken, a turkey, a duck, a goose, a quail, a pheasant, a ratite, an ornamental
bird or a feral bird.

61. The transgenic avian of Claim 51, wherein the avian is a chicken.

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